Characterization of tomato genetic resources in the function of breeding

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Summary: Tomato is one of the most important species belonging to the Solanaceae family. Focusing on the importance of tomato in human nutrition and the problem of narrowed genetic variability, the aim of the study was to assess morphological and chemical diversity in IFVCNS germplasm collection. Twenty genotypes were analysed for the morphological and chemical fruit traits: average mass (g), length (cm), diameter (cm), pericarp thickness (mm), locules number, moisture content (%), total soluble solids (°Brix), ash content (%), total acidity (%) and pH value. Selected plant material for analysis included: landraces, traditional varieties, breeding lines and commercial varieties. Differences among tomato genotypes in all fruit traits were determined. Fruit mass and locules number had the highest coefficient of variation. The least differences between genotypes were observed in the fruit moisture content. Four principal components accounted for 90.6% of total variance or 36.5%, 24.2%, 19.8% and 10.1%, respectively. Along the axis of the first main component, genotypes were classified into three groups. The first component was defined by fruit length, diameter and mass. The second component was correlated with pericarp thickness and locules number, and the third with moisture content, ash content and total soluble solids. Based on the cluster analysis, genotypes were classified into three groups which were in agreement with the PCA groups. Hybridization between genotypes from different groups was proposed in order to create new hybrids and varieties and to increase tomato germplasm diversity. By crossing those genotypes, improved recombinations in morphological and chemical traits can be expected.

Keywords: breeding, diversity, fruit traits, genetic resources, genotypes, germplasm collection, germplasm diversity, Solanum lycopersicum, tomato

Introduction

Tomato (Solanum lycopersicum L.) is a very important part in varied and balanced diet consumed in numerous ways, from fresh state to industrial products. Presence of tomatoes in a human diet is very important because of the content of minerals, vitamins, proteins, essential amino acids, carotenoids (lycopene and β-carotenoids), monounsaturated fatty acids, and phenolic compounds, many of which have antioxidant effects (Ali et al., 2021). Although it does not have a high nutritional value, high consumption makes it one of the main sources of vitamins and minerals in the human diet. Significance of tomato is reflected by the rising trend of harvested areas and production in recent decades. According to the FAO (Food and Agriculture Organization of the United Nations) statistical data, in the period 1999-2019 world’s harvested areas (ha) increased by more than 21% and production (t) by more than 39% (http://www.fao.org/statistics).

Although there are almost 10,000 tomato cultivars today, large worldwide producers use only a small fraction of these (Castellana et al., 2020). In addition to the already narrowed genetic variability, initially caused by the bottleneck, further impoverishment of tomato genetic resources and loss of desirable genes is inevitable. Plant genetic diversity is a very important prerequisite to cope with climate changes and withstand an attack of diseases and pests (Paulauskas et al., 2013). Reduction of genetic diversity leads to the permanent
disappearance of many genes from the genetic pool, and loss of numerous traditional varieties. Since genetic erosion in numerous crops is now a common phenomenon, characterization, preservation and enhancement of diversity in existing resources are of global importance. Vegetable Department of the Institute of Field and Vegetable Crops (IFVCNS) in Novi Sad, Serbia, contributes to the conservation of genetic resources of vegetables by maintaining germplasm collections of a large number of vegetable species, including tomatoes. Different kinds of plant materials are included in the collections such as wild relatives, landraces, traditional and commercial varieties, hybrids and breeding lines. Passport data were collected for genotypes in collections, including morphological, chemical and biochemical traits. Notable genetic loss due to breeding was detected in the modern tomato gene pool (Gonias et al., 2019). The constant striving to increase the yield and tolerance to abiotic and biotic stress, as well as to improve the quality of the fruit, imposes the need to create new genotypes with characteristics that exceed the existing assortment. As an important prerequisite for achieving this goal is the diversity of the initial material from which the selection of potential parental pairs will be made. Tomato landraces and traditional varieties are of particular importance (Henareh et al., 2015; Wang et al., 2017). As valuable gene pools, landraces evolved under artificial and natural selection pressure, contain useful allele combinations that are not present in modern varieties (Marin-Montes et al., 2016). Being one of the main goals of tomato breeding nowadays, fruit quality is determined by numerous chemical and morphological traits. Among chemical compounds, the most important are total soluble solids (TSS or °Brix, g sucrose/100 g sample), and volatile compounds (Alonso et al., 2009). TSS content is a very important indicator of technological quality because it leads to increase yield and reduce dehydration costs during processing (Amor & Amor, 2007), with great influence on fruit flavour. Although mineral matter (ash content) represents a small percentage of dry matter content, it significantly affects the nutritive value and quality of fruit and tomato products (Dias, 2012). Content of organic acids influence the flavour and the storage of processed tomatoes, especially citric acid (Wilkerson et al., 2013). For processing varieties, pH value is a peculiarly important indicator of the susceptibility of industrial products to the attack of microorganisms. Lower pH values inhibit the activity of bacteria Bacillus coagulans by reducing the number of spores (Vercammen et al., 2012) and decrease the probability of deterioration of the products due to the presence of thermophilic organisms. Consumers also assess the fruit quality based on fruit size, especially uniformity. The presence of genotypes of different fruit size on the market is necessary because of differences in consumer preferences, production methods and purposes. Another important morphological trait for the quality is pericarp thickness, since pericarp and internal dividing walls have the highest dry matter content. Thicker pericarp along with the firm skin and flesh enables longer storage and improve the preservation of harvested fruits (Yeboah et al., 2014). Therefore, the improvement of all those chemical parameters is of great importance in the creation of new hybrids and varieties intended for fresh consumption and industrial processing as well.

Given the above, the aim of this study was to 1) assess the diversity of 20 tomato genotypes based on morphological and chemical fruit traits, 2) classify tomato genotypes based on similarities and differences in analysed fruit traits, and 3) determine potential genotypes for hybridisation to create new hybrids and varieties and to increase tomato germplasm diversity.

Material and Methods

Germplasm collection and field trials

Twenty genotypes from IFVCNS tomato germplasm collection were selected for diversity assessment. Plant material included: landraces, traditional varieties, breeding lines and commercial varieties (Table 1). All breeding lines, commercial varieties, and landrace S99 originated from Serbia. Four landraces (S13, S29, S70 and S78) originated from North Macedonia. Among selected genotypes, there were two American (S340 and S320) and one French (S338) traditional variety, while one was of unknown origin (S359).

The experiment was set up in three consecutive years (2010-2012) on the experimental fields at Rimski šančevi site (45°20’ N, 19°51’ E, 84 m). Sowing was conducted in the mixture of burnt manure: soil (1:1) at the beginning of April. When the seedlings were in cotyledons phase, they were transferred on the same substrate with 10 × 10 cm plant space. The seedlings were planted in May on the open field in a completely randomized block design, with five replications, two rows and ten plants per row. Row spacing was 70 cm while space between plants within a row was 50 cm. In each replication, one fruit per plant was harvested, from ten randomly chosen plants. Fruits were picked from the first inflorescence and analysed for the following traits: average mass (g), length (cm), diameter (cm), pericarp thickness (mm), and locules
number. Fruit length, diameter and pericarp thickness were measured using a calliper. Average fruit mass was determined by dividing fruit yield per plot by the number of fruits per plot.

Chemical analyses

Chemical analyses were performed in the second year of the experiment at the Institute of Food Technology, University of Novi Sad, Serbia. Fruits for analysis were harvested at full maturity, from the first inflorescence. To form a representative sample, a few randomly selected fruits from each replication were picked, homogenized in a kitchen blender (Bosch, Germany), rapidly frozen and stored in a refrigerator at -18 °C prior to analysis. The following parameters were investigated:

1. Moisture content (%) and ash content (%) were determined using a thermogravimetric analyser (TGA701, LECO Corporation, USA). Moisture content was determined by drying samples at 105 °C to constant weight. After drying, the sample was incinerated at 900 °C to constant weight, to determine ash content.

2. Total soluble solids content (g sucrose/100 g sample, °Brix) was read directly on the refractometer (ATR ST Plus, Schmidt + Haensch, Germany).

3. Total acidity (g citric acid monohydrate/100 g of sample) was determined by titration with sodium hydroxide solution in the presence of phenolphthalein. The sample was filtered through coarse filter paper, and total acidity was determined from the filtrate. Titration with NaOH solution was conducted till the occurrence of a light pink colour, stable for 30 s (AOAC, 2000).

4. pH value was measured directly from the sample, using a pH meter with the temperature probe for temperature correction (Denver Instrument, USA).

Data analysis

For each trait, arithmetic mean as the central tendency indicator was calculated. To determine the dispersion of traits, standard deviation and coefficient of variation were calculated. Principal component analysis (PCA) was performed to assess a contribution of particular traits to total variability and to classify genotypes into groups. To verify similarity with PCA grouping, hierarchical cluster analysis was performed for grouping genotypes based on analysed traits using UPGMA (Unweighted Pair-Group Method with Arithmetic mean) method and the Euclidean distance (Sokal & Michener, 1958). Statistical software StatSoft, Inc. (2013) STATISTICA v. 12. was used for data processing.

Results and discussion

Descriptive statistics

Variability in all traits was determined (Table 1). The lowest fruit length (3.8 cm) was found in landrace S99, while the highest value (6.3 cm) was measured in the commercial variety Bačka. Henareh et al. (2015) reported variation in fruit length from 2.5 cm to 7.5 cm in hundred tomato genotypes. In our study, landrace S78 had the lowest fruit diameter and mass (4.2 cm and 55.8 g), while the highest values of those traits were found in the traditional variety S320 (7.6 cm and 207 g). In the study of Campos de Melo et al. (2015) the smallest fruit had mass of 17.5 g and the largest had 95.6 g. Bearing in mind that fruit length, diameter and average mass are main yield components and selection criteria for improving tomato yield, those traits were subject of numerous studies (Hernández- Bautista et al., 2015; Tošić et al., 2018). Fruits with more locules number generally have flattened shape (Rodriguez et al., 2011) which was the case in this study, since the greatest locules number (11.2) was found in genotype with most flattened shape, landrace S99 (Fig. 1).

The lowest locules number (2.1) was found in landrace S29. According to Wang et al. (2017), variation in locules number ranged from 2 to 19, while accessions with two and three locules constituted 41%. Pericarp thickness exhibited variation among genotypes from 3.6 mm in landrace S99 to 7.8 mm measured in breeding line V18 (Fig. 1). Wang et al. (2017) reported higher range of variation, from 1.77 mm to 8.98 mm. Fruits with thicker pericarp can withstand shipping and remain firm for longer periods and thus help in reducing postharvest losses.
Table 1. Descriptive statistics for analysed traits of 20 tomato genotypes

<table>
<thead>
<tr>
<th>No</th>
<th>Genotype</th>
<th>FL (cm)</th>
<th>FD (cm)</th>
<th>PT (mm)</th>
<th>LN</th>
<th>FM (g)</th>
<th>MC (%)</th>
<th>AC (%)</th>
<th>TSS (°Brix)</th>
<th>pH</th>
<th>TA (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>S 340</td>
<td>5.6</td>
<td>6.8</td>
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<td>174.3</td>
<td>93.1</td>
<td>0.31</td>
<td>6.3</td>
<td>4.5</td>
<td>0.31</td>
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<td>S 359</td>
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<td>5.2</td>
<td>175.8</td>
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<td>0.37</td>
<td>5.7</td>
<td>4.5</td>
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</tr>
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<td>S 338</td>
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<td>5.6</td>
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<td>0.39</td>
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<td>55.8</td>
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<td>0.42</td>
<td>5.4</td>
<td>4.3</td>
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<tr>
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<td>93.1</td>
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<td>10</td>
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<td>5.8</td>
<td>171.5</td>
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<td>0.21</td>
<td>4.5</td>
<td>4.4</td>
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<tr>
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<td>V 9</td>
<td>5.3</td>
<td>6.7</td>
<td>5.3</td>
<td>5.7</td>
<td>158.3</td>
<td>92.8</td>
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<td>6.2</td>
<td>4.5</td>
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<td>V 18</td>
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<td>5.1</td>
<td>7.8</td>
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<td>92.7</td>
<td>0.32</td>
<td>5.9</td>
<td>4.4</td>
<td>0.34</td>
</tr>
<tr>
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<td>V 21</td>
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<td>5.8</td>
<td>5.7</td>
<td>4.4</td>
<td>107.3</td>
<td>93.2</td>
<td>0.37</td>
<td>5.4</td>
<td>4.3</td>
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<tr>
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<td>O 3</td>
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<td>5.7</td>
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<td>5.4</td>
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<td>122.7</td>
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<td>O 15</td>
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<td>5.7</td>
<td>5.4</td>
<td>4.6</td>
<td>116.1</td>
<td>92.6</td>
<td>0.41</td>
<td>6.2</td>
<td>4.6</td>
<td>0.45</td>
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</table>

The lowest moisture content (92.6%) was measured in the breeding line O15 and the traditional variety S338, while the highest (94.3%) was measured in the commercial variety Bačka. TSS varied from 4.5°Brix in variety Bačka to 6.3°Brix in the traditional variety S340. TSS values of 6°Brix and above were measured in breeding lines O10, O15, O14, O15, and V9, the traditional variety S338, the commercial variety Knjaž and the landrace S13. Henareh et al. (2015) reported similar TSS variation from 3.4 to 6.8. Evaluating fruit quality indicators in some traditional varieties grown in Tunisia, Aoun et al. (2013) reported TSS content from 2.02°Brix to 4.57°Brix. The lowest ash content in our study (0.21%) was found in the commercial variety Bačka, while the highest (0.42%) were determined in landrace S78 and the traditional variety S338. These results are in agreement with Dubofuor et al. (2010). The lowest pH value (4.2) was measured in landrace S99, while the highest (4.7) was determined in traditional variety S338 and landrace S13. Pal et al. (2018) reported higher range of pH values, from 3.9 to 5.08, in twenty-two tomato cultivars. TA varied from 0.28% in variety Bačka to 0.51% in breeding line O14. Measures of dispersion indicated the existence of diversity in the examined plant material. The highest coefficient of variation was determined for locules number (37.5%) and fruit mass (34.4%). Mehta & Asati (2008) also detected the highest coefficient of variation for fruit mass and number of locules, and emphasized the possibility of obtaining higher selection response for these traits. Campos de Melo et al. (2015) reported the highest coefficient of variation for the number of fruits per plant and locules number. Variability in fruit mass is attributed to the presence of 28 QTLs (Quantitative Trait Locus) (Grandillo et al., 1999). The same authors highlighted that the allelic variations within four loci (fw1.1, fw2.2, fw3.1 and fw4.1) influence fruit mass, with differences up to 30%. Moisture content had the lowest coefficient of variation in our material (0.5%), indicating the greatest uniformity of genotypes in this trait.

Classification of genotypes by PCA

PCA reduced ten initially observed variables into four artificial, uncorrelated variables, or main components which accounted for most of the variance in the observed variables (Table 2).
Four components accounted for 90.6% of the total variance. The individual contribution of each of the four components in total variability was 36.5%, 24.2%, 19.8% and 10.1%, respectively. As a criterion for the separation of variables which were largely defined isolated components served the values of factor loading > 0.70. The first component was defined by fruit length, fruit diameter, and fruit mass. The second component was largely correlated with pericarp thickness and locules number, and the third with moisture content, ash content and total soluble solids. The fourth component, that explained the lowest % of variability, was defined by pH value. Genotypes projection on a surface covered by the first two components was shown with the aim of visual presentation and data interpretation (Fig. 2).

Along the axis of the first main component, majority of genotypes, except three, were classified into three groups. Inside the first factor, genotypes were grouped according to fruit length, diameter and average mass. Bernousi et al. (2011) also found that variables showing high correlation with PC1 can be considered as representatives of fruit size. The results agree with Henareh et al. (2015) where the first three components accounted for 71.6% of total variability, among 100 tomato genotypes. The same authors came to the conclusion that yield and yield components also highly correlated with the first component, which indicates that they might be used as selection criteria in breeding programs aimed to identify genotypes with high yield. Having in mind that the information about the variation among tomato landrace populations is still limited, Sacco et al. (2015) screened a wide diversity of tomato genotypes with different geographical origins. Results of the PCA indicated that population were grouped mainly based on fruit mass and locules number.

The first group of genotypes (I) in this study includes four landraces (S78, S29, S70, S13) and one breeding line O13 (Fig. 2). They had on average, the smallest fruit mass (72 g), length (4.2 cm), diameter (4.9 cm) and the number of locules (3.6). All landraces from this group originate from North Macedonia and could have the same genetic background. The second (II) group consists of genotypes with medium fruit size (on average 123.5 g). It includes four breeding lines from Serbia (O10, O14, O15 and V21) and French traditional variety Saint Pierre (S338). This group had on average the highest values of TSS (5.94 °Brix) and TA (0.44%). Breeding lines O10, O14 and O15 have one common parent which can be considered as a reason for being in the same group. The third (III) group has seven genotypes and included commercial varieties (Bačka and Knjaz), breeding lines (V9 and O3) and traditional varieties (S340, S359 and S320). On average, this group had the highest fruit mass (171.7 g), length (5.8 cm), diameter (6.8 cm) and locules number (5.7). However, in this group pericarp thickness (5.4 mm), ash content (0.33%) and acidity (0.37%) had the lowest values. Commercial varieties (Bačka and Knjaz) and breeding lines (O3 and V9) from this group originated from Serbia, while two (S320 and S340) of three traditional varieties are from the USA.

It can be noticed that genotypes from Serbia were found in all formed groups. Numerous authors concluded that genotypes of the same origin were distributed in different groups, which may be a consequence of different selection pressure. Therefore, these authors emphasized that in choosing genotypes for crossing, in order to obtain new gene recombination, preference should be given to genetic over geographical diversity (Pawar et al., 2013; Meena & Bahadur, 2015). Also, numerous studies emphasized that genotypes of different origin can be found in the same group, which is proved to be true in the case of our study, since no group includes only genotypes of the same origin. Those studies further led to the conclusion that there does not have to be parallelism between genetic diversity and geographical origin (Kumar et al., 2013).

Some landraces from this study had low fruit size and high TSS content, but there were also varieties or lines with larger fruits and high TSS content, improved through breeding. This leads to the conclusion that fruit mass should not be a limiting factor in breeding focused on increasing the dry matter content. Three genotypes were observed

<table>
<thead>
<tr>
<th>Factor</th>
<th>Eigenvalue (λ)</th>
<th>% Total variance</th>
<th>Cumulative Eigenvalue</th>
<th>Cumulative %</th>
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<td>2.424</td>
<td>24.238</td>
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<td>1.006</td>
<td>10.057</td>
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</table>
outside of the formed groups because of their specificity in some of the traits. The breeding line V18 had the thickest pericarp (7.8 mm), while the variety Alparac had the lowest TSS (4.71). Landrace S99 was the most distinct from all genotypes since it had the lowest values of fruit length (3.8 cm) and pericarp thickness (3.6 mm), and the highest locules number (11.2). All landraces were grouped together, except one (S99). Although most of the breeding lines were classified in the second group, some lines were also found in the other two groups. The majority of varieties belonged to the third group. Landraces from this study had the lowest fruit size and average mass, and were hence grouped together. Those landraces originate from North Macedonia and probably, as mentioned above, have the same genetic background.

For centuries, farmers have been carrying out distinct selections in different cultivation areas that resulted in numerous ecotypes, or landraces, adapted to different environmental conditions (Cebolla-Cornejo et al., 2013). One of the first breeding goals was to increase fruit mass and yield. All varieties from this study had higher fruit size and mass compared to landraces. On the other hand, modern breeding strives to satisfy different consumers and processing industry demands by making improved and new varieties. Lines and commercial varieties from this study were created for different purposes and type of production, and different market demands, which may explain their presence in all groups. These results indicated that PCA points up specific traits of interest for practical breeding purposes and enables identification of promising genotypes for tomato breeding.

**Classification of genotypes by cluster analysis**

According to the cluster analysis, genotypes were classified into three groups (Fig. 3). Group I included all genotypes from PCA III and the traditional variety S338 from PCA II. Group II consisted of all genotypes from PCA II except one (S338) and included S99 and V18, which were outside of three formed PCA groups.

All genotypes from PCA I were grouped together in Group III plus variety Alparac. Since these two analyses have different theoretical bases, the established similarity in the grouping can serve as an empirical measure of accuracy (Laurentin & Karlovsky, 2006). The importance of genotypes

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**Figure 2.** Projection of 20 tomato genotypes on surface covered by two main components

**Figure 3.** Dendrogram of 20 tomato genotypes based on analysed fruit traits
grouping by applying appropriate statistical methods is reflected in directing breeders in the breeding process and saving their time. Genotypes are classified into groups (clusters) in such a way that the intra-group variability is less than intergroup variability. Potential parents for crossing are selected from the most distant groups (clusters). Hybridization between genotypes from different cluster and PCA groups can be recommended to create new recombination and to increase tomato germplasm diversity. By crossing appropriate genotypes, new lines and hybrids with new recombination and improved morphological and chemical characteristics can be expected.

Conclusion

Analysed tomato genotypes differed in all morphological and chemical fruit traits. PCA enabled segregation of variables that have largely contributed to the differentiation of analysed material and caused genotypes classification into three groups. Composition of three groups obtained by cluster analysis was in agreement with the PCA groups. Hybridization of genotypes from appropriate groups was suggested to create new hybrids and varieties and to increase tomato germplasm diversity. Genotypes with the thickest pericarp, highest TSS, ash content and acidity were identified, as promising for quality improvement. By increasing the pericarp thickness and TSS content, preservation of harvested fruits would be improved and the taste and quality of products. Also, the costs of dehydration during processing would be reduced and factory yield increased.

References


Karakterizacija genetičkih resursa paradaža u funkciji oplemenjivanja

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Sažetak: Paradajz je jedna od najznačajnijih vrsta iz familije Solanaceae. Fokusirajući se na značaj paradajza u ljudskoj ishrani i problem njegove sužene genetske varijabilnosti, cilj istraživanja je bio da se proceni morfološki i hemijski diverzitet u kolekciji germplazme Instituta za ratarstvo i povrtarstvo u Novom Sadu. Dvadeset genotipova je odabrano za analizu sledećih osobina ploda: prosečna masa (g), dužina (cm), širina (cm), debljina perikarpa (mm), broj komora, sadržaj vode (%), ukupna rastvorljiva suva materija (°Brix), sadržaj pepela (%), ukupna kiselost (%). Biljni materijal odabran za analizu obuhvatao je: lokalne populacije, stare sorte, oplemenjivačke grupa. Ukrštanjem tih genotipova mogu se očekivati unapređene rekombinacije u morfološkim i hemijskim osobinama. Prosečna masa ploda i broj komora imali su najveći koeficijent varijacije. Ispitivani genotipovi se najmanje razlikovali u dužini i komercijalne sorte. Utvrđene su razlike između genotipova u svim ispitivanim osobinama. Prosečna masa ploda je odabrano za analizu sledećih osobina ploda: prosečna masa (g), dužina (cm), širina (cm), debljina perikarpa (mm), broj komora, sadržaj vode (%), ukupna rastvorljiva suva materija (°Brix), sadržaj pepela (%), ukupna rastvorljiva suva materija (°Brix), sadržaj pepela (%), ukupna kiselost (%). Biljni materijal odabran za analizu obuhvatao je: lokalne populacije, stare sorte, oplemenjivačke grupa. Ukrštanjem tih genotipova mogu se očekivati unapređene rekombinacije u morfološkim i hemijskim osobinama.

Ključne reči: diverzitet, diverzitet germplazme, genetički resursi, genotipovi, kolekcija germplazme, oplemenjivanje, osobine ploda, paradajz, Solanum lycopersicum

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